

WHAT IS CLAIMED IS:

1 1. A method of determining total urokinase concentration in a sample containing either
2 or both active and inactive forms of urokinase, comprising:

3 obtaining a first peptide corresponding to a sequence in Seq. ID No. 16
4 between amino acid residues 1 and 135;

5 obtaining a second peptide corresponding to a sequence in Seq. ID No. 16
6 between amino acid residues 159 and 411;

7 obtaining a third peptide corresponding to a sequence in Seq. ID No. 16
8 which includes amino acid residues 158 and 159;

9 obtaining an immunological composition directed against each of said
10 peptides;

11 contacting aliquots of said sample with each of said immunological
12 compositions; and,

13 determining the quantity of each of said immunological compositions which
14 is bound to at least one of said forms of urokinase in each of said aliquots.

1 2. The method of claim 1 wherein said first peptide is one as in any of Seq. ID Nos. 1-
2 6.

- 1 3. The method of claim 1 wherein said second peptide is one as in any of Seq. ID Nos.
2 7-12.

- 1 4. The method of claim 1 wherein said third peptide is one as in any of Seq. ID No. 13-
2 14.

- 1 5. The method of claim 1 wherein each of said immunological compositions has a
2 binding affinity constant for said peptide from which it is derived that is
3 substantially higher than its binding affinity constant for a non-urokinase protein as
4 similar in amino acid sequence to urokinase as is trypsin.

- 1 6. The method of claim 1 wherein said third peptide is one as in Seq. ID No. 14, and is
2 used to derive an immunological composition which exhibits a binding affinity
3 constant for urokinase zymogen of at least $1 \times 10^8 \text{ M}^{-1}$, and a binding affinity
4 constant for forms of urokinase lacking a peptide bond between amino acid residues
5 158 and 159 of Seq. ID No. 16 of at least approximately 10-fold lower than that for
6 urokinase zymogen.

- 1 7. The method of claim 1 wherein an additional peptide corresponding to a sequence in
2 Seq. ID No. 16 which includes amino acid residues 135 and 136 is obtained and
3 used to derive said immunological composition.

- 1 8. The method of claim 7 wherein said additional peptide is that of Seq. ID No. 17.

- 1 9. The method of claim 1 wherein the immunological composition is an antiserum, an
2 antibody, a hybridoma, or a supernatant of a hybridoma, obtained via injection into a
3 mammal of said peptide.

- 1 10. The method of claim 1 wherein said determination of said quantity of each of said
2 immunological compositions is carried out by radiolabeling said sample or said
3 immunological composition.

- 1 11. The method of claim 1 wherein said determination of said quantity of each of said
2 immunological compositions is carried out by:
 - 3 determining the amount of low molecular weight urokinase in said sample
4 which binds to one or more of said immunological compositions directed against
5 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
6 residues 159 and 411, but which does not bind to one or more of said
7 immunological compositions directed against said peptide corresponding to a
8 sequence in Seq. ID No. 16 between amino acid residues 1 and 135;

 - 9 determining the amount of high molecular weight urokinase in said sample

10 which binds to one or more of said immunological compositions directed against
11 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
12 residues 1 and 135 or between amino acid residues 159 and 411, but which does not
13 bind to one or more of said immunological compositions directed against said
14 peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid
15 residues 158 and 159; and,

16 determining the amount of urokinase zymogen in said sample which binds
17 to one or more of said immunological compositions directed against said peptide
18 corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues
19 158 and 159.

1 12. The method of claim 1 wherein said determination of said quantity of each of said
2 immunological compositions is carried out by:

3 determining the amount of low molecular weight urokinase in said sample
4 which binds to one or more of said immunological compositions directed against
5 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
6 residues 159 and 411, but which does not bind to one or more of said
7 immunological compositions directed against a peptide corresponding to Seq. ID
8 No. 17;

9 determining the amount of high molecular weight urokinase in said sample
10 which binds to one or more of said immunological compositions directed against
11 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
12 residues 1 and 135 or between amino acid residues 159 and 411, but which does not

13 bind to one or more of said immunological compositions directed against said
14 peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid
15 residues 158 and 159; and,

16 determining the amount of urokinase zymogen in said sample which binds
17 to one or more of said immunological compositions directed against said peptide
18 corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues
19 158 and 159.

1 13. A method of determining total urokinase concentration in a sample containing either
2 or both active and inactive forms of urokinase, comprising:

3 obtaining a peptide as in any of Seq. ID Nos. 1-6;

4 obtaining a peptide as in any of Seq. ID Nos. 7-12;

5 obtaining a peptide as in any of Seq. ID No. 13-14;

6 obtaining an immunological composition selected from the group of
7 immunological compositions consisting of an antisera, an antibody, a hybridoma, or
8 a supernatant of a hybridoma, said immunological compositions each obtained via
9 injection into a mammal of said peptide;

10 contacting aliquots of said sample with each of said immunological
11 compositions; and,

12 determining the quantity of each of said immunological compositions which
13 is bound to at least one of said forms of urokinase in each of said aliquots, wherein
14 said determination of the quantity of each of said immunological compositions is
15 carried out by:

16 determining the amount of low molecular weight urokinase in said sample
17 which binds to one or more of said immunological compositions directed against
18 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
19 residues 159 and 411, but which does not bind to one or more of said
20 immunological compositions directed against said peptide corresponding to a
21 sequence in Seq. ID No. 16 between amino acid residues 1 and 135;

22 determining the amount of high molecular weight urokinase in said sample
23 which binds to one or more of said immunological compositions directed against
24 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
25 residues 1 and 135 or between amino acid residues 159 and 411, but which does not
26 bind to one or more of said immunological compositions directed against said
27 peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid
28 residues 158 and 159; and,

29 determining the amount of urokinase zymogen in said sample which binds
30 to one or more of said immunological compositions directed against said peptide
31 corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues
32 158 and 159.

1 14. A kit for determining total urokinase concentration in a sample containing either or

2 both active and inactive forms of urokinase, comprising:

3 immunological compositions, selected from the group of immunological
4 compositions consisting of an antisera, an antibody, a hybridoma, or a supernatant
5 of a hybridoma;

6 said immunological compositions each obtained via injection into a mammal
7 of a peptide directed against each of a set of peptides including: a first peptide
8 corresponding to a sequence in Seq. ID No. 16 between amino acid residues 1 and
9 135, a second peptide corresponding to a sequence in Seq. ID No. 16 between
10 amino acid residues 159 and 411, and a third peptide corresponding to a sequence in
11 Seq. ID No. 16 which includes amino acid residues 158 and 159; and,

12 instructions for contacting aliquots of said sample with each of said
13 immunological compositions, and determining the quantity of each of said
14 immunological compositions which is bound to at least one of said forms of
15 urokinase in each of said aliquots.

1 15. The kit of claim 14 wherein said first peptide is one as in any of Seq. ID Nos. 1-6.

1 16. The kit of claim 14 wherein said second peptide is one as in any of Seq. ID Nos. 7-
2 12.

1 17. The kit of claim 14 wherein said third peptide is one as in any of Seq. ID No. 13-14.

10 determine the amount of low molecular weight urokinase in said sample
11 which binds to one or more of said immunological compositions directed against
12 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
13 residues 159 and 411, but which does not bind to one or more of said
14 immunological compositions directed against said peptide corresponding to a
15 sequence in Seq. ID No. 16 between amino acid residues 1 and 135;

16 determine the amount of high molecular weight urokinase in said sample
17 which binds to one or more of said immunological compositions directed against
18 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid
19 residues 1 and 135 or between amino acid residues 159 and 411, but which does not
20 bind to one or more of said immunological compositions directed against said
21 peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid
22 residues 158 and 159; and,

23 determine the amount of urokinase zymogen in said sample which binds to
24 one or more of said immunological compositions directed against said peptide
25 corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues
26 158 and 159.

- 1 22. A peptide for determining total urokinase concentration in a sample as in any of
2 Seq. ID Nos. 1-15 and 17.

- 1 23. An antisera obtained via injection into a mammal of a peptide for determining total

2 urokinase concentration in a sample as in any of Seq. ID Nos. 1-15 and 17.

1 24. An antibody obtained via injection into a mammal of a peptide for determining total
2 urokinase concentration in a sample as in any of Seq. ID Nos. 1-15 and 17.

1 25. A hybridoma producing an antibody obtained via injection into a mammal of a
2 peptide for determining total urokinase concentration in a sample as in any of Seq.
3 ID Nos. 1-15 and 17.

1 26. A supernatant of a hybridoma producing an antibody obtained via injection into a
2 mammal of a peptide for determining total urokinase concentration in a sample as in
3 any of Seq. ID Nos. 1-15 and 17.